pg 1

Final Report
for
CRADA No. ORNL94-~~JYS-152~~ 445.02
with
Formally Union Camp Corporation (Now International Paper Co.)

Overcoming Constraints to High-Yield Plantation-Grown Hardwoods in the Southeastern United States

Project Overview

This project was comprised of the following four inter-related tasks.

Task 1 - Plantation Maintenance and Measurement

Data on dry weight productivity per tree and/or growth as measured by individual tree height and diameter at a specified height on the stem was determined at the end of each of five years corresponding to ages 2 through 6. Measurements of height and diameter were recorded once a month during the growing season on a subsample of four trees per clone per species per treatment combination. Dry biomass in the leaf litter traps during the growing season once the canopy has closed was periodically collected and measured. Foliar nutrient levels were determined once a month by removing LPI 8 on each subsampled measurement tree and completing nutrient analyses. Weather data, including precipitation, minimum and maximum temperature and photosynthetically active radiation on an hourly basis were recorded daily. Information on irrigation rates and fertilization levels were collected.

Task 2 - Intra- And Interspecific Variation In Osmotic Potential

The specific objectives of this task were:

(1) to determine whether limitation in water availability constrains productivity and influences leaf osmotic potential of cottonwood, sycamore, and/or sweetgum growing under short-rotation field conditions,

(2) to document the occurrence of osmotic adjustment under varying levels of water availability levels, and

(3) to determine the effect of nitrogen fertilization on osmotic potential and response to irrigation.

Task 3 - Leaf Gas Exchange And Water-Use Efficiency

The specific objectives of this task were:

(1) to quantify the contribution of photosynthesis, respiration, and water-use efficiency to the productivity of individual cottonwood, sycamore, and sweetgum trees grown under various levels of water and/or nutrient availability, and

(2) to quantify intra- and interspecific variability for photosynthesis, respiration, and water-use efficiency for cottonwood, sycamore, and/or sweetgum.

Task 4 - Whole-Plant Carbon Budgets

The specific objectives of this task were:

(1) to evaluate foliar and non-foliar dry matter allocation with respect to water and/or nutrient availability,

(2) to test the impacts of water and/or nutrient availability on tissue-specific respiration rates, and

(3) to evaluate whole-plant carbon budgets for individual clones or species as a means of determining the relative limitations placed on above-ground production by respiratory processes in branches, stems, and roots.

Project Summary

Task 1 - Plantation Maintenance and Measurement

Overall Experimental Design and Treatment Levels –

The experiment was established in 1995 on 3.0 ha within the Trice Experimental Forest, owned and operated by Union Camp Corp., south of Mayesville, SC (37° 59' 40" N, 56° 88'58" E) within the Black River watershed primarily containing the Norfolk and Goldsboro soil series (Fig. 1). Prior to this experiment the land had been in continuous agriculture, with soybeans being the most recent crop. Unrooted cuttings from 8 eastern cottonwood (*Populus deltoides* Bartr.) clones [designated R, W, B, Y, RB, WB, BB and YB], bare root seedlings of a single open-pollinated seed lot of American sycamore (*Platanus occidentalis* L.) and rooted cutting of 4 sweetgum (*Liquidambar styraciflua* L.) clones [designated R, W, B and Y] were planted at a 2.5 x 3.0 m spacing adjacent to a drip irrigation emitter. Weeds were controlled for the first two years by shallow tilling and directed hand spraying with RoundUp. Insects (primarily cottonwood leaf beetle) were controlled periodically as needed throughout the life of the experiment using a combination of insecticides.

Each species received one of five treatment combinations assigned to three randomized blocks. Each species + treatment + replication combination occupied approximately 0.2 ha. There were two irrigation levels and two fertilization levels

established in a 2x2 factorial design, plus a Non-Irrigated 'Control' treatment which received no irrigation and the low level of fertilization, for total of five treatments.

Irrigation treatments in 1996, 1997 and 1998 were based on time domain reflectometry (TDR, TRASE Systems, Soil Moisture Equipment, Inc., Santa Barbara, CA) reading taken 3-5 times per week from plots distributed across species, replications and treatments. Trees were watered via drip tube emitters located every 1 m within the rows at a delivery rate of four l m^{-1}. The target volumetric soil water content was 10% and 15%, respectively for the Low and High Irrigation treatments. The High Irrigation treatments were rewatered when the available soil moisture was depleted by 30%; the Low Irrigation treatments were rewatered when the available soil moisture was depleted by 15%. The target soil volumetric soil water content was based on empirically derived soil moisture release curves for each soil series. Irrigation treatments in 1999 and 2000 were based on a weekly average increment distributed throughout the 28-week growing season. The Low Irrigation treatment received approximately 30% less water than the High Irrigation treatment. Thus, irrigation treatments varied by species and by year. The cumulative irrigation levels and precipitation levels for each species and each year are presented in Table 1.

In an effort to match fertilizer applications to plant growth and demand, fertilization treatments were varied by species and by year. In all cases, fertilizer was delivered by two means, an initial granular application of diammonium phosphate, at 20% of the annual elemental nitrogen fertilizer prescription and a weekly intermittent application of the remnant 80% annual elemental nitrogen prescription via the drip irrigation system throughout the remainder of each growing season (Table 2). The fertigation application provided the remainder of the nitrogen prescription along with all other macro and micronutrients in the ratio of 10% N to 0% P to 5% K to 1% Ca to 0.5% Mg to 0.01% Cu to 0.05% Mn to 0.01% Mo to 0.03% Zn to 0.02% B in the form of ammonium nitrate, potassium nitrate, calcium nitrate, magnesium nitrate, potassium chloride, copper sulfate, manganese sulfate, zinc sulfate, sodium molybdate and boric acid. This prescription was based on preliminary soil samples distributed across the site. Based on these soil samples it was determined that iron and phosphorus were not limiting on the site. The resulting nitrogen prescriptions in kg N/ha by species, by treatment, by year are presented in Table 2. The Low Fertilizer treatment received one half the prescription for total elemental nitrogen of the High Fertilizer treatment as well as a proportional reduction of all other elements. The Non-Irrigated Control received the Low Fertilizer treatment.

Irrigation and fertilizer treatment combinations are thus designated as Treatment 1 for low irrigation and low fertilization, Treatment 2 for low irrigation and high fertilization, Treatment 3 for high irrigation and low fertilization, Treatment 4 for high irrigation and high fertilization and Control for no irrigation and low fertilization.

Among the 16-24 trees per block in cottonwood, the 100 trees per block in sycamore and the 25 trees per block in sweetgum, four trees per treatment per block were selected as permanent measurement trees. For each of these trees diameters was recorded at breast height starting in the second year on a weekly based on dendroband readings. Monthly leaf nutrient samples were collected throughout the growing season in year 2, in May, July and October in year 3, and in July in year 4. Our target leaf nitrogen concentration was 3% (dry weight: dry weight) or greater on the High Fertilizer treated

trees. Leaf area indices, based on distributed litter traps, were estimated in years 2, 3 and 4. And, end of season height and diameter measures were measured on all trees in all years. In addition to these standing-tree measurements, in 1996, 1997, 1998, 1999 and 2000, years 2 through 6, individual trees from the border rows surrounding the permanent measurement trees were destructively sampled to determine biomass (i.e., dry weight) of each component part (i.e., stem, limb, leaf and root biomass). Sampled trees were cut at ground line, segregated into their component parts, weighed in the field and then subsampled to determine fresh weight to dry weight ratios for each component part. Root biomass was estimated in 1998, 1999, and 2000 based on soil auger sampling surrounding the removed tree. Eight 18-cm^2 soil cores to a depth of 1 m were removed from around each sampled tree at preset variable distances from the tree. These cores were separated into three depth classes: 0 to16 cm, 17 to 50 cm and 51 to 100 cm. For each depth class the soil was sieved through a 1-cm screen and the remaining roots were separated into three size categories: < 2 mm diameter, 2 mm to 1 cm diameter, and > 1 cm diameter. These roots were then dried and weighed to determine root dry weight per representative soil volume. Finally, in 1998 and 1999, the root/stump ball was sampled using a mechanical tree spade to remove a 1-m diameter cone of soil encompassing the original tree. These root balls were rinsed clean in the field and air-dried over several months to determine the dry weight of the coarse root directly below the tree.

Climatic data was also recorded on site. Hourly mean, maximum and minimum temperature, mean, maximum and minimum water vapor pressure, maximum and minimum wind speed, photosynthetically active solar radiation and cumulative potential evapotranspiration, and cumulative daily rainfall entered into an electronic data logger for each growing season.

Data from the cottonwood and sweetgum portion of the experiment was analyzed using a nested factorial experiment in a randomized complete block design using a mixed model ANOVA, with block and trees as random effects and treatments and clones as fixed effects. Single-degree-of-freedom orthogonal contrasts were used to test treatment differences contrasting control vs. all other treatments, High Irrigation vs. Low Irrigation, and High Fertilizer vs. Low Fertilizer. When treatment or clone differences were detected in the ANOVA, a Waller-Duncan k-ration t-test was used to separate means. Data from the sycamore portion of the experiment was analyzed using a nested experiment in a randomized complete block design using a mixed model ANOVA, with block and seedlings within blocks as random effects and treatments as a fixed effect. Single-degree-of-freedom orthogonal contrasts were used to test treatment differences contrasting control vs. all other treatments, High Irrigation vs. Low Irrigation, and High Fertilizer vs. Low Fertilizer. When treatment differences were detected in the ANOVA, a Waller-Duncan k-ration t-test was used to separate means. All tests were performed at $p \leq 0.05$.

Results

Cottonwood Growth and Performance

Averaged across all clones, replications and treatments, leaf nitrogen concentration (on a % dry weight basis) peaked early in the growing season and then

same over years 3, 4, 5 and 6. Late-season LAI was always lower than mid-season LAI and averaged around 2.00 m^2/m^2.

Height and diameter varied significantly throughout the length of the experiment (Fig. 13 & 14). Generally the non-irrigated control was the poorest performing treatment though this was not true for height in years 5 and 6. The two high fertilizer treatments experienced poorer height growth relative to the two low fertilizer treatments from year s2 on, to the point where they were not significantly difference from the control in the last two years of the study. The same response occurred in diameter tough only first detectable in year 4 and never to the point where diameter in the high fertilizer treatments equaled the poorer performance of the control treatment. By year 6, the best treatment for height growth was the low water, low fertilization treatment and the best treatment for diameter growth was either of the low fertilization treatments. It is likely, based on the height and diameter data and the noted changes in soil chemistry (Fig. 9), that the initial pulse application at the beginning of each growing season of diammonium phosphate was having a cumulative negative impact on soil pH, nutrient availability, and possibly meristematic and cambial growth.

Height and diameter also varied significantly by clones throughout the length of the experiment (Fig. 15 & 16). The R clone performed the best for both height and diameter at all ages; the W and Y clones were consistently the poorest performers. There were significant cone by treatment interactions, for both height and diameter, though this was due mainly to changes in the relative treatment effect, that is, clone ranks were generally the same within each treatment. The best clone by treatment combination by the end of the study was the R clone under treatment 1 for height and clone R under either treatment 1 or 3 for diameter.

As expected, total tree and component (i.e., stem, limb and leaf) biomass varied significantly with treatment and reflected differences in the standing tree measurements (Fig. 17). There were large differences in biomass between the non-irrigated control and all other treatments at each age and for each component. The best clone by treatment combination also produced measurably larger amounts of biomass at each age and for each component. On a total above ground basis, leaf biomass did not increase after year 2 and became proportionally smaller over time. At the end of the experiment, the best clone by treatment combination produced an average of 47 kg of stem dry weight per tree, 16 kg of limb biomass per tree and a last season annual leaf component of 2.7 kg per tree.

Sampled root biomass increased between years 4 and 6 (Fig. 18). The majority of the sampled root biomass occurred in the 0-15 cm sampling depth (51%), followed by the 16-45 cm depth (29%), and then by the 46-100 cm depth (21%). Though not significant due to the high variability among samples, there was a trend for higher amounts of root biomass under the higher irrigation treatments, as expressed as root dry weight per 200 ml of sampled soil to a depth of 15 cm (Fig 18). The negative high fertilizer effect noted in the above ground biomass components was not evident in the below ground biomass. Finally, the greatest amount of below ground biomass on a per unit volume of soil basis, independent of clone or treatment, occurred in the excavated root ball.

The cottonwood clones examined in this study generally performed as well as or better than other *Populus* clones tested in other regions of the U.S. through age 3 (Fig. 19). In year 3, crown closure was complete, LAI peaked and incremental height and diameter growth began to decline. By year 6, the best clone by treatment combination in

this study performed at an intermediate level relative to other studies. Irrigation and fertilization did not appear to allow the tested clones to perform as well as the best clones in the Pacific Northwest, though irrigation and fertilization did enhance growth within the study itself. The largest difference among the studies is the degree of genetic improvement in the tested plant materials. The clones in the current study represent clonal replicates of selected wild germplasm. Alternatively, the material in the Pacific Northwest came from a hybrid-breeding program.

The only early rotation variables, including irrigation and fertilization treatment levels, that were significantly correlated with year 6 height or diameter, were leaf level boron and zinc concentrations. Leaf level boron concentrations in year 3 were positively correlated with height and diameter in year 6 ($r=0.54$ and $r=0.61$, respectively). Leaf level zinc concentrations in year 3 were negatively correlated with height and diameter in year 6 ($r=-0.65$ and $r=-0.41$, respectively).

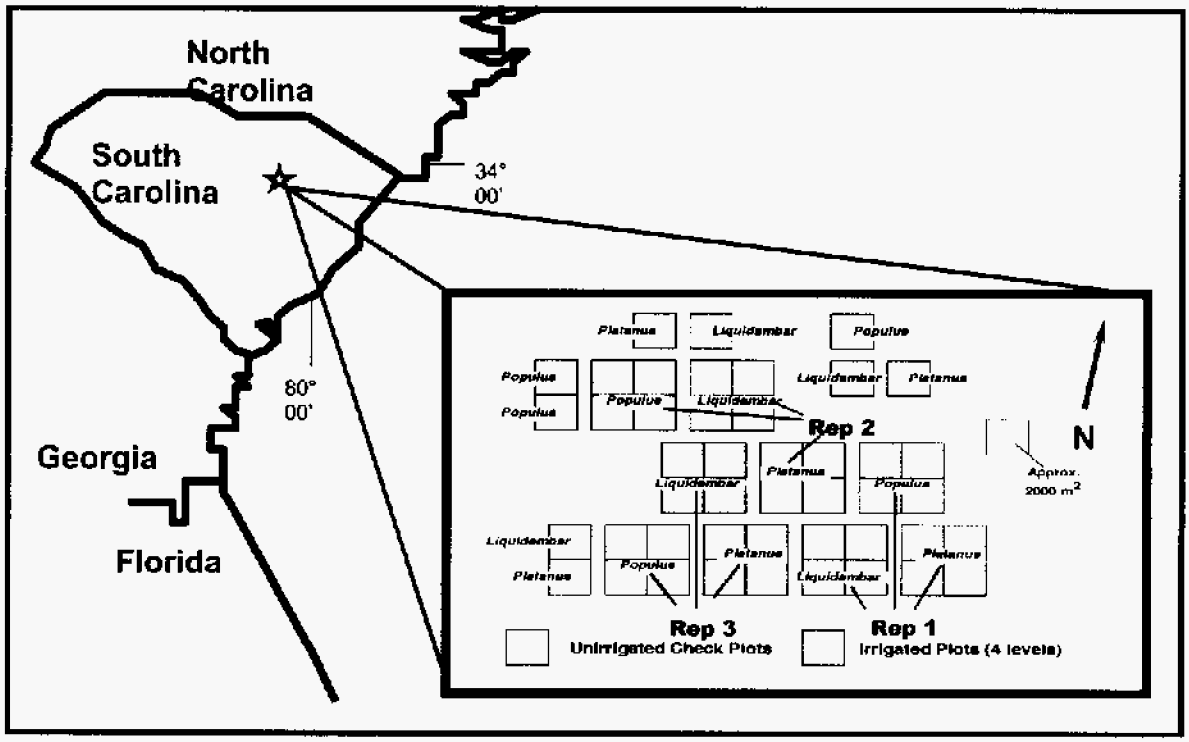

Figure 1. Geographic location and experimental field design for a 2 x 2 + 1, fertilizer x irrigation + control fertigation trial established in 1995 in Sumter Co., SC, USA.

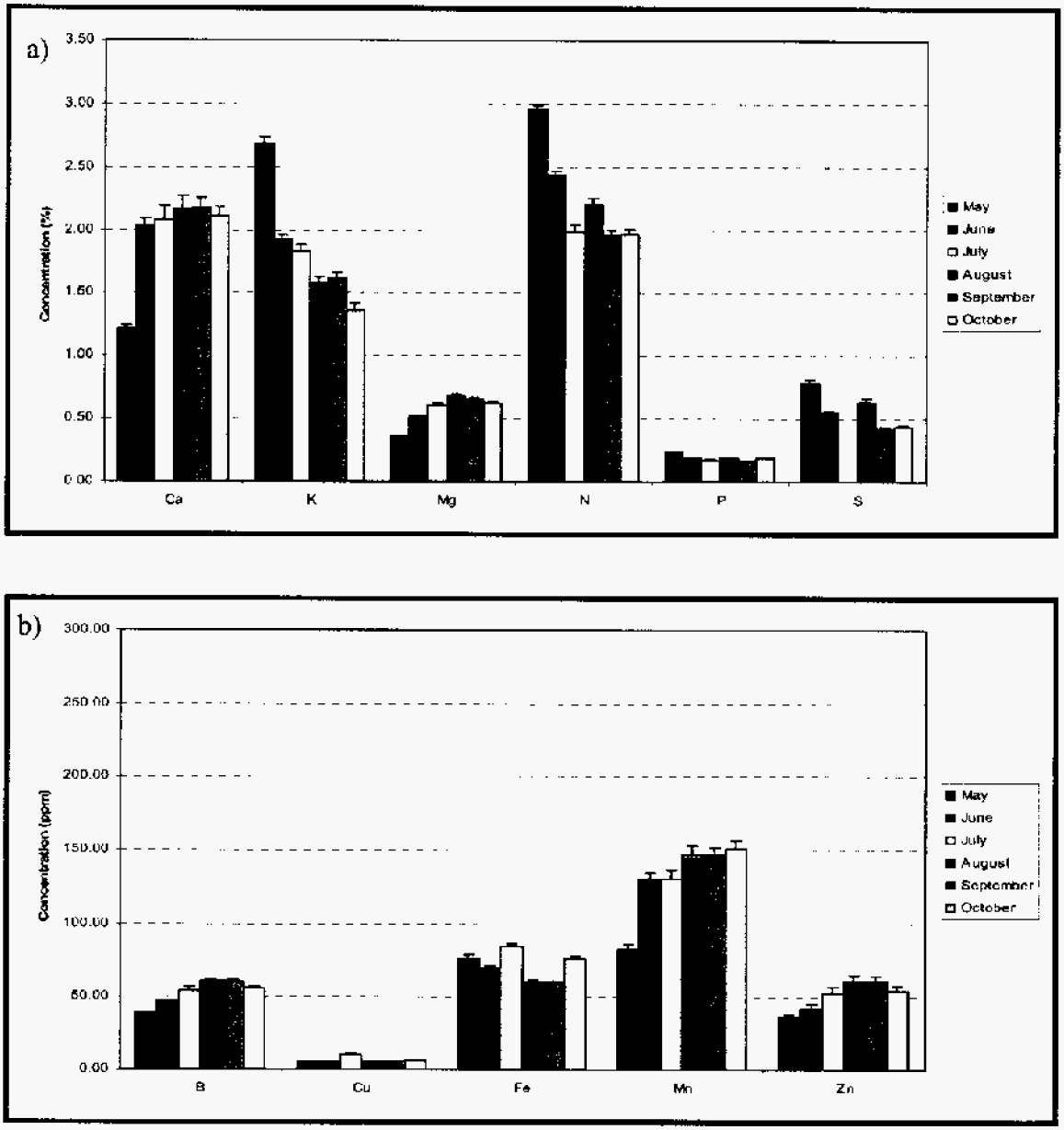

Figure 2. Changes during the growing season in leaf macro and micronutrient levels in 2-year-old cottonwood grown in a short-rotation intensive culture fertigation experiment near Sumter, SC. Horizontal bars on each treatment mean designate the standard error of the mean.

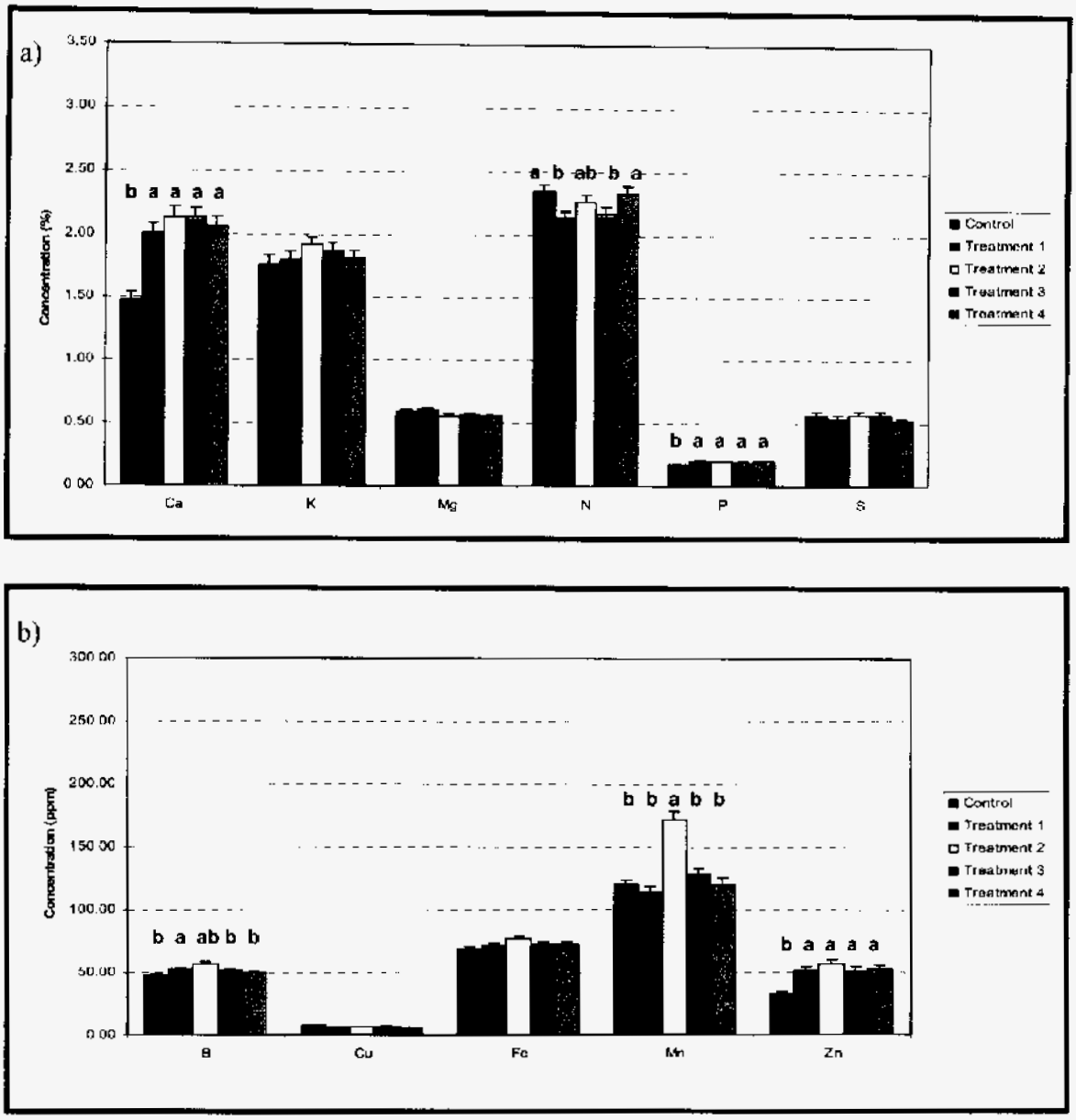

Figure 3. Mean treatment differences in leaf macro and micronutrient levels in 2-year-old cottonwood grown in a short-rotation intensive culture fertigation experiment near Sumter, SC. Treatment 1 = low irrigation and low fertilization; Treatment 2 = low irrigation and high fertilization, Treatment 3 = high irrigation and low fertilization, Treatment 4 = high irrigation and high fertilization, and Control = no irrigation and low fertilization (See Tables 1 and 2 for annual levels of irrigation and fertilization). Horizontal bars on each treatment mean designate the standard error of the mean. Means within nutrients not connected by the same letter are significantly different at $p \leq 0.05$ based on Waller-Duncan's k-ratio t-test.

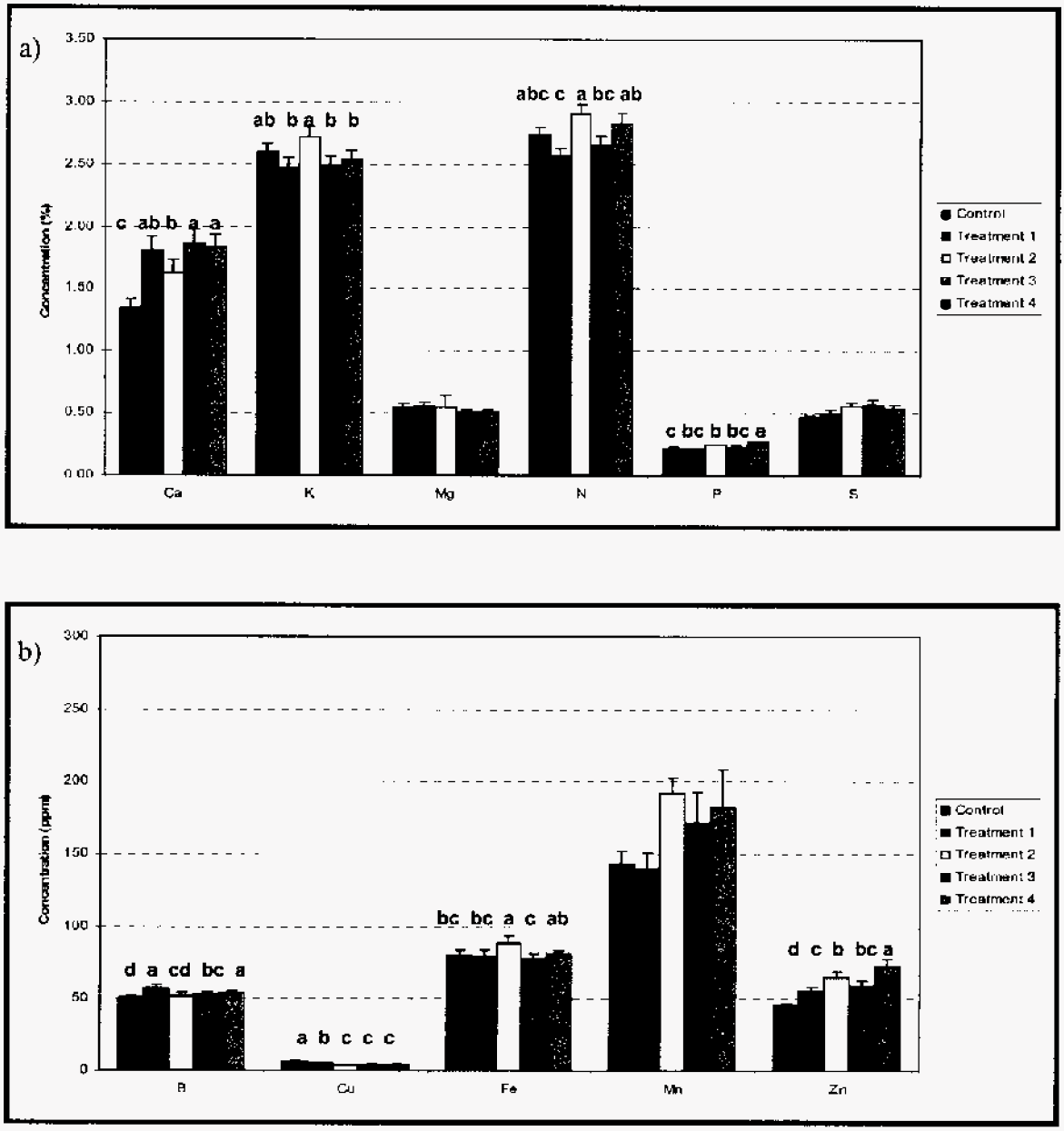

Figure 4. Mean treatment differences in leaf macro and micronutrient levels in 3-year-old cottonwood grown in a short-rotation intensive culture fertigation experiment near Sumter, SC. Treatment 1 = low irrigation and low fertilization; Treatment 2 = low irrigation and high fertilization, Treatment 3 = high irrigation and low fertilization, Treatment 4 = high irrigation and high fertilization, and Control = no irrigation and low fertilization (See Tables 1 and 2 for annual levels of irrigation and fertilization). Horizontal bars on each treatment mean designate the standard error of the mean. Means within nutrients not connected by the same letter are significantly different at $p \leq 0.05$ based on Waller-Duncan's k-ratio t-test.

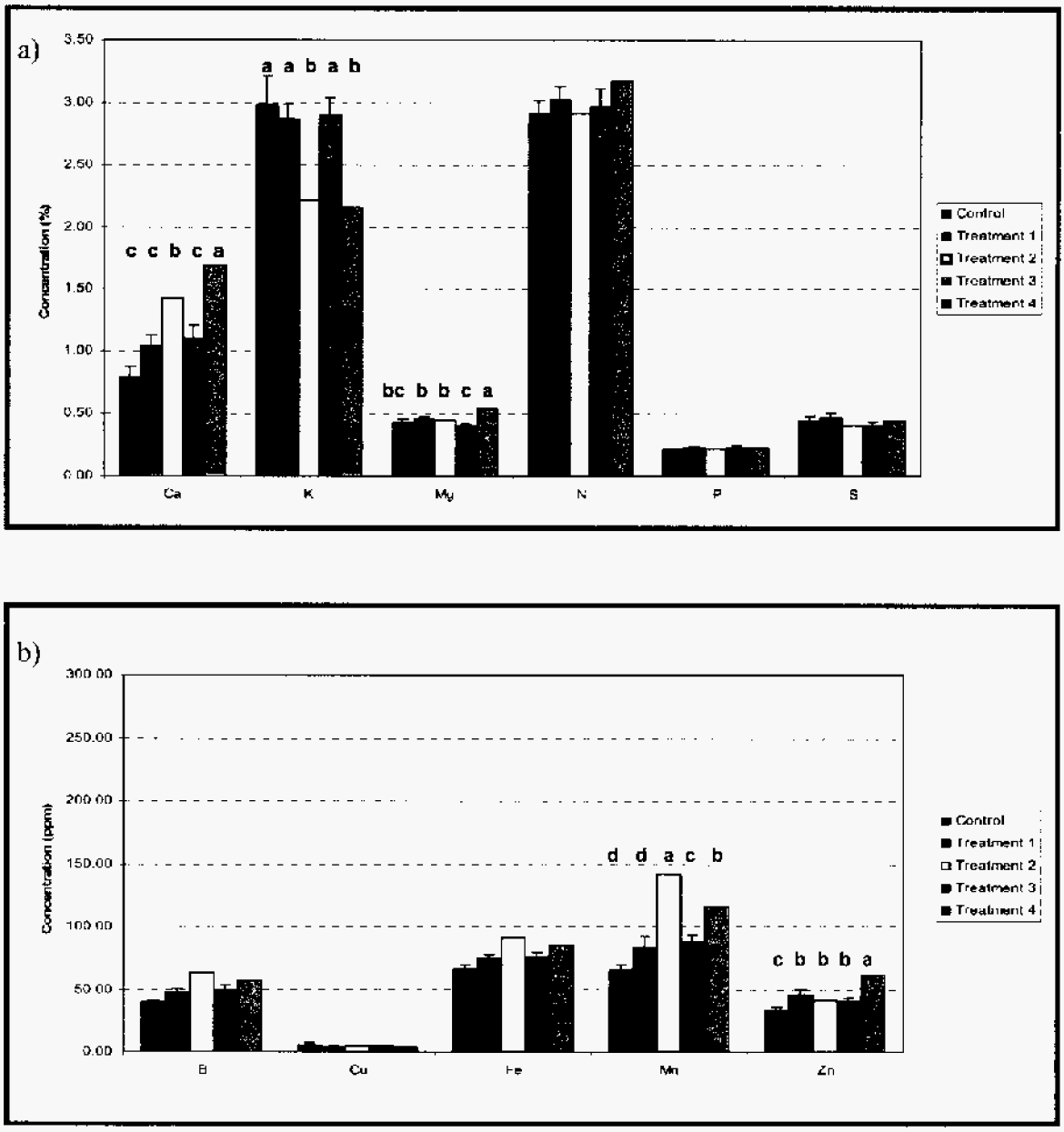

Figure 5. Mean treatment differences in leaf macro and micronutrient levels in 4-year-old cottonwood grown in a short-rotation intensive culture fertigation experiment near Sumter, SC. Treatment 1 = low irrigation and low fertilization; Treatment 2 = low irrigation and high fertilization, Treatment 3 = high irrigation and low fertilization, Treatment 4 = high irrigation and high fertilization, and Control – no irrigation and low fertilization (See Tables 1 and 2 for annual levels of irrigation and fertilization). Horizontal bars on each treatment mean designate the standard error of the mean. Means within nutrients not connected by the same letter are significantly different at $p \leq 0.05$ based on Waller-Duncan's k-ratio t-test.

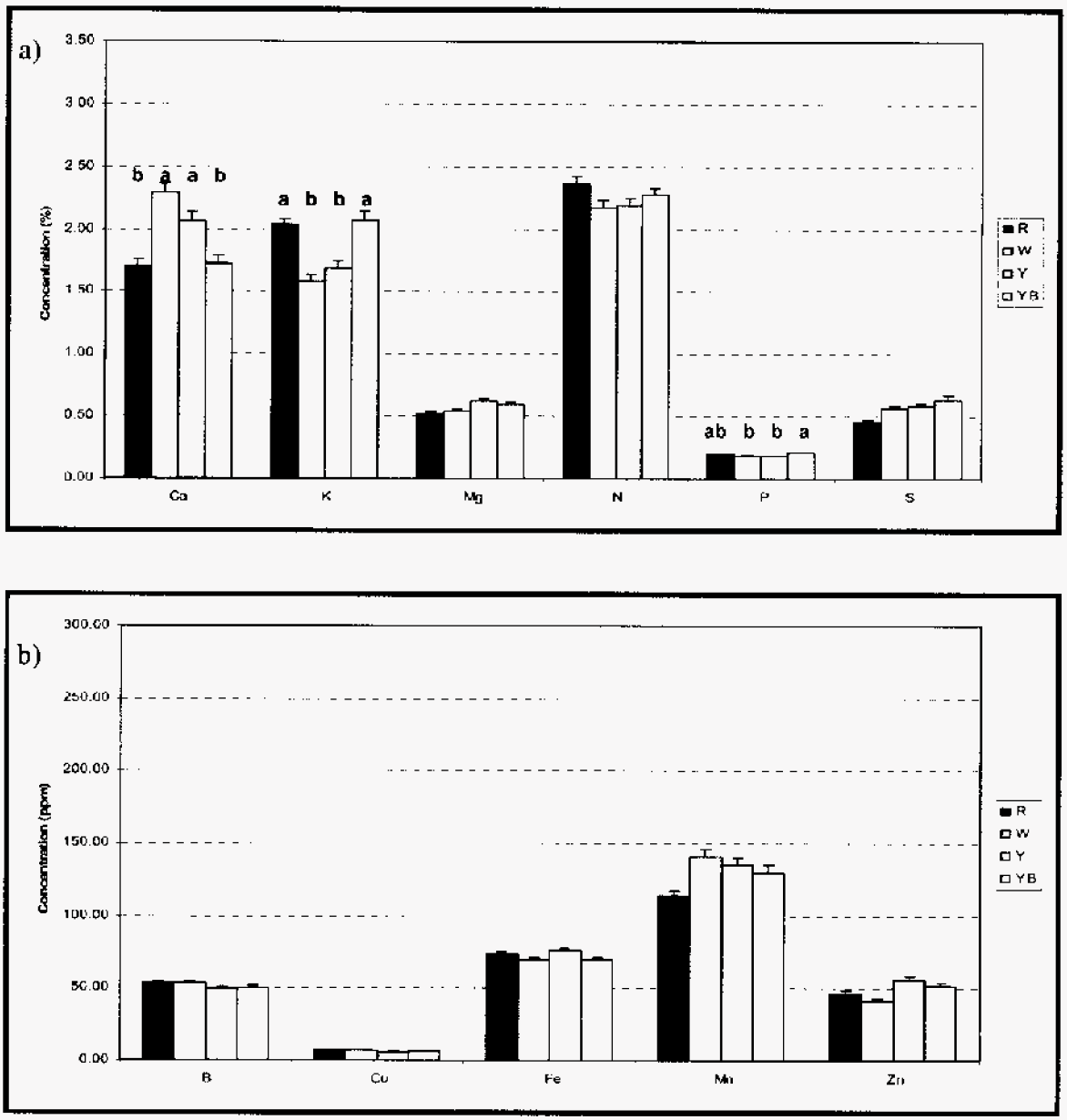

Figure 6. Mean clonal differences in leaf macro and micronutrient levels in 2-year-old cottonwood grown in a short-rotation intensive culture fertigation experiment near Sumter, SC. Horizontal bars on each treatment mean designate the standard error of the mean. Means within nutrients not connected by the same letter are significantly different at $p \leq 0.05$ based on Waller-Duncan's k-ratio t-test.

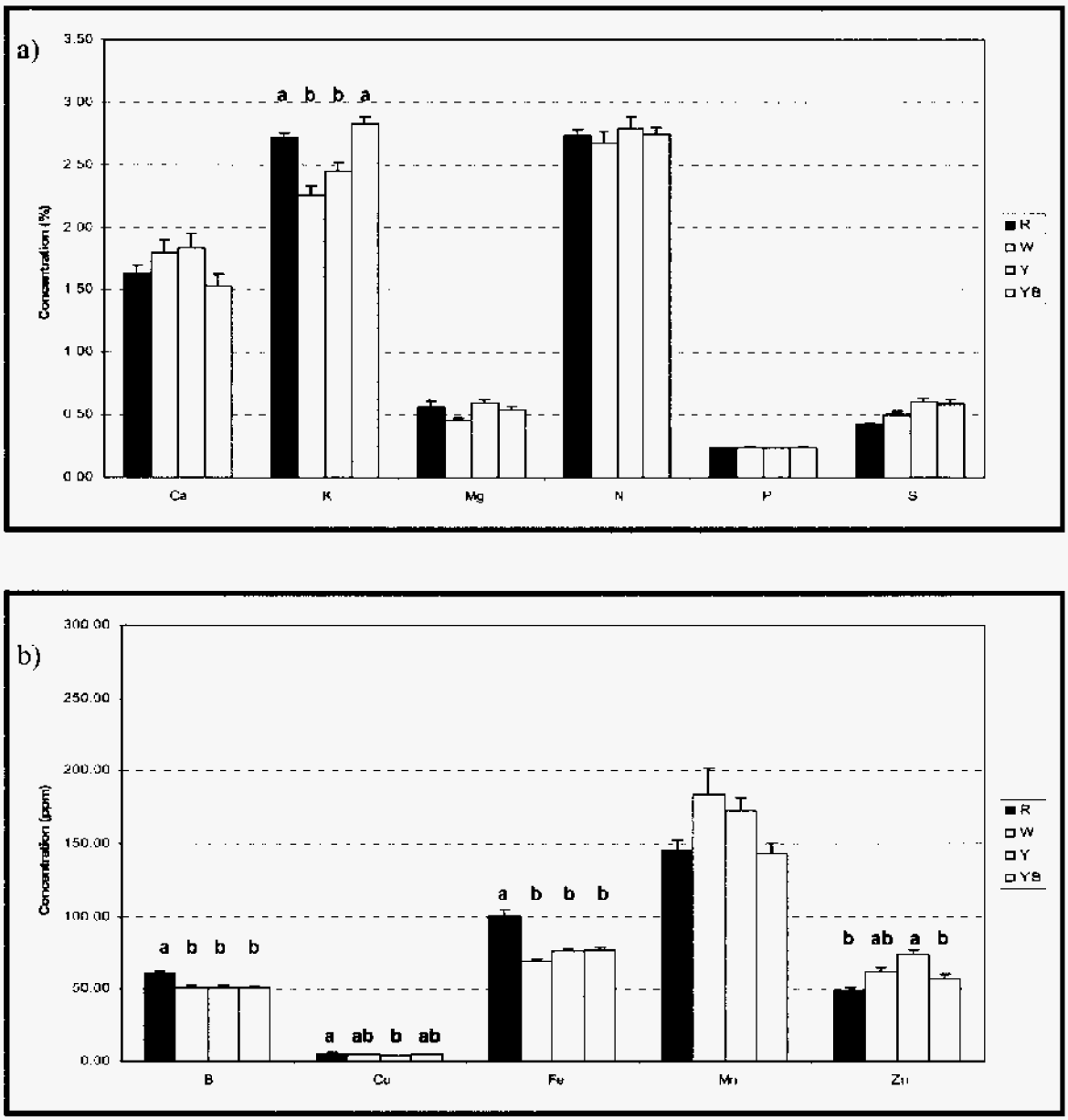

Figure 7. Mean clonal differences in leaf macro and micronutrient levels in 3-year-old cottonwood grown in a short-rotation intensive culture fertigation experiment near Sumter, SC. Horizontal bars on each treatment mean designate the standard error of the mean. Means within nutrients not connected by the same letter are significantly different at $p \leq 0.05$ based on Waller-Duncan's k-ratio t-test.

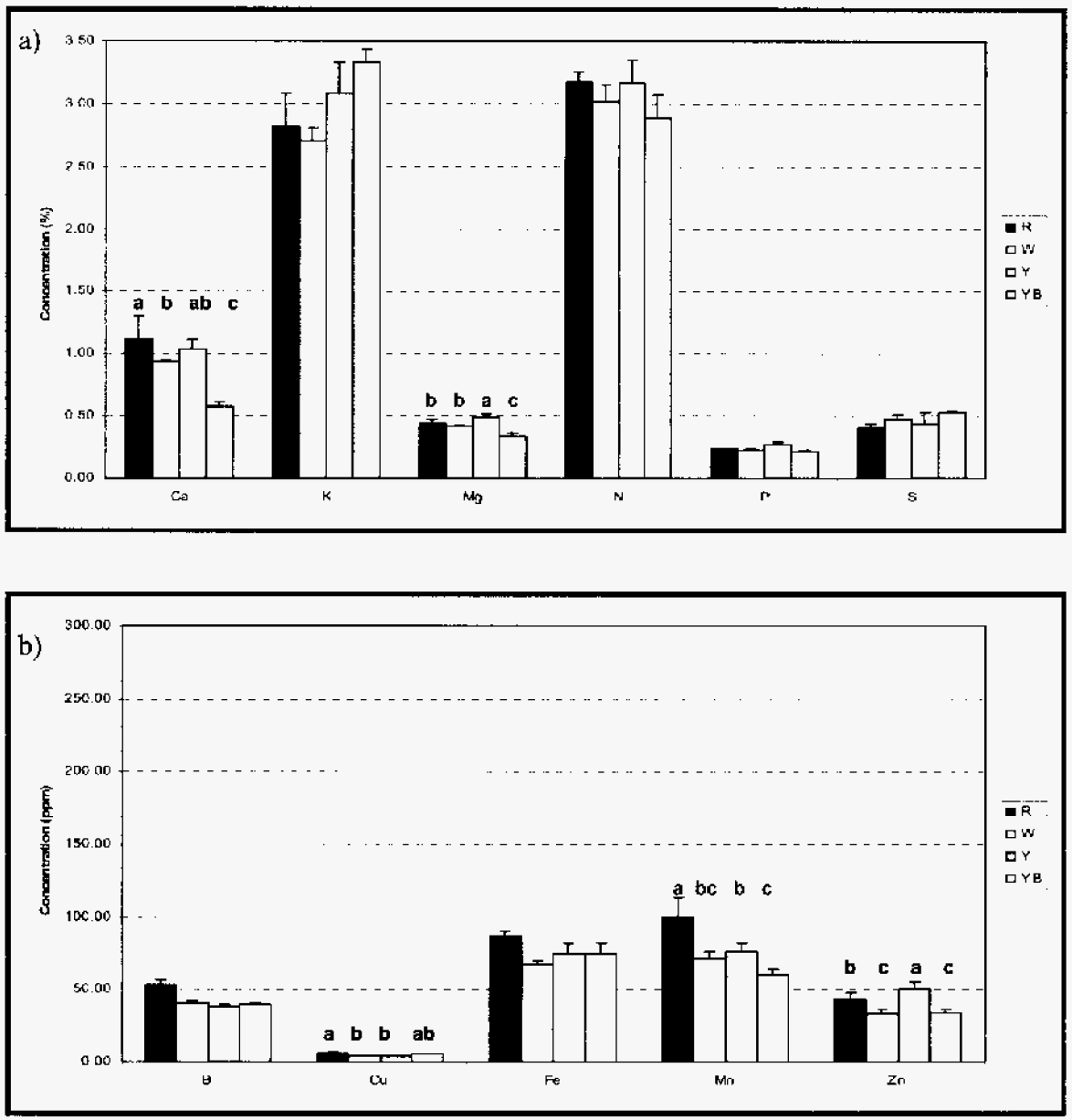

Figure 8. Mean clonal differences in leaf macro and micronutrient levels in 4-year-old cottonwood grown in a short-rotation intensive culture fertigation experiment near Sumter, SC. Horizontal bars on each treatment mean designate the standard error of the mean. Means within nutrients not connected by the same letter are significantly different at $p \leq 0.05$ based on Waller-Duncan's k-ratio t-test.

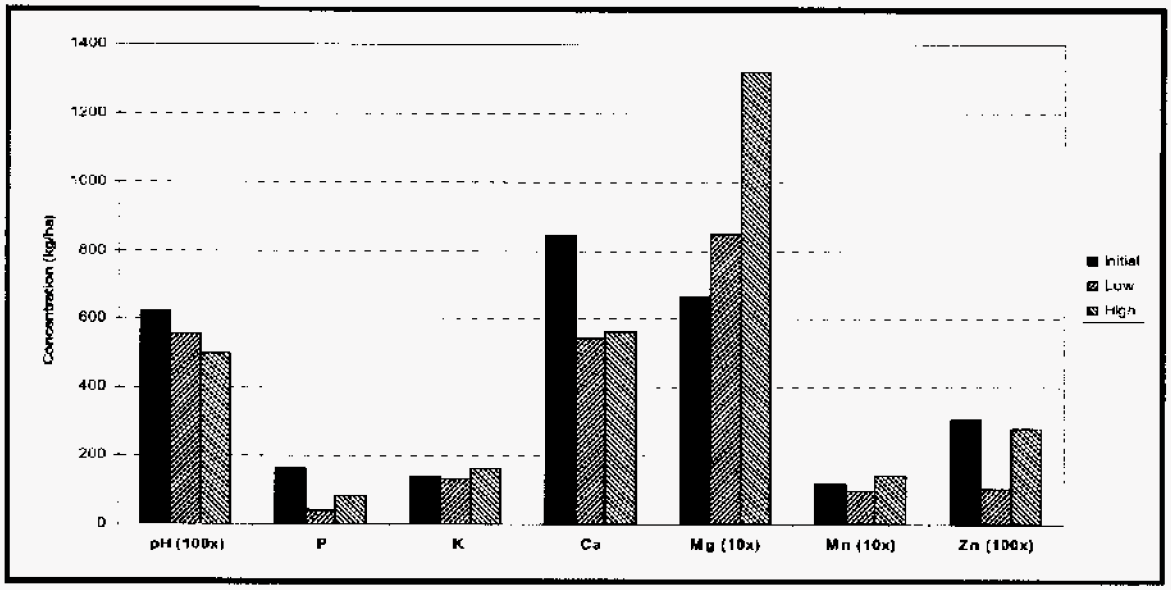

Figure 9. Changes in soil chemistry under 5-year-old cottonwood grown in a short-rotation intensive culture fertigation experiment near Sumter, SC. See Tables 1 and 2 for a description of the low and high fertilization treatment levels.

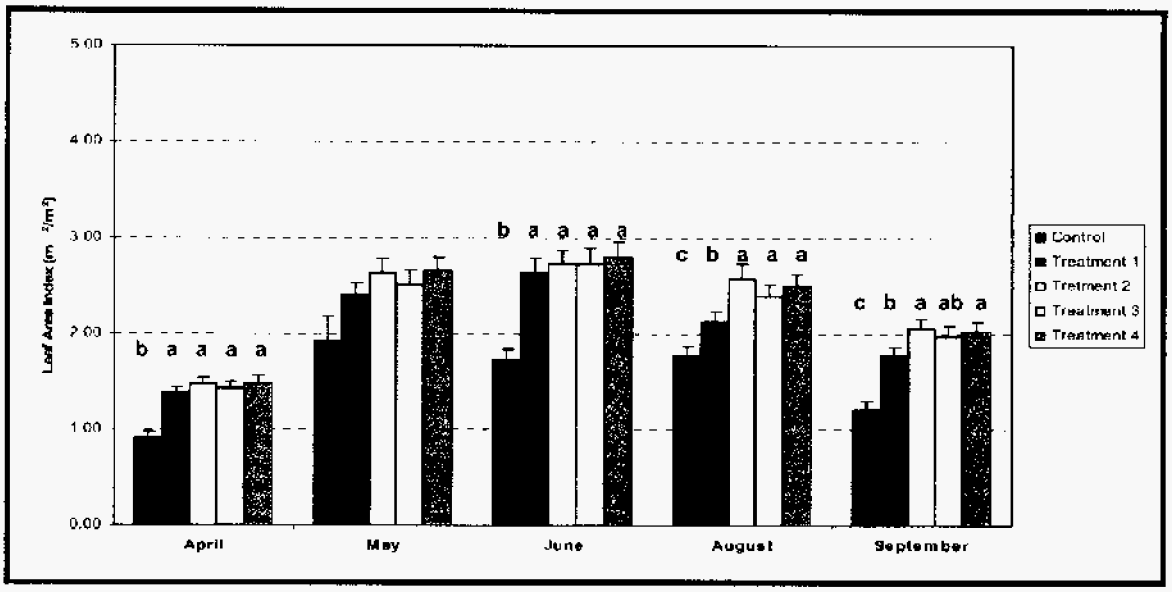

Figure 10. Mean treatment differences in leaf area index in 3-year-old cottonwood grown in a short-rotation intensive culture fertigation experiment near Sumter, SC. Treatment 1 = low irrigation and low fertilization; Treatment 2 = low irrigation and high fertilization, Treatment 3 = high irrigation and low fertilization, Treatment 4 = high irrigation and high fertilization, and Control = no irrigation and low fertilization (See Tables 1 and 2 for annual levels of irrigation and fertilization). Horizontal bars on each treatment mean designate the standard error of the mean. Means within nutrients not connected by the same letter are significantly different at $p \leq 0.05$ based on Waller-Duncan's k-ratio t-test.

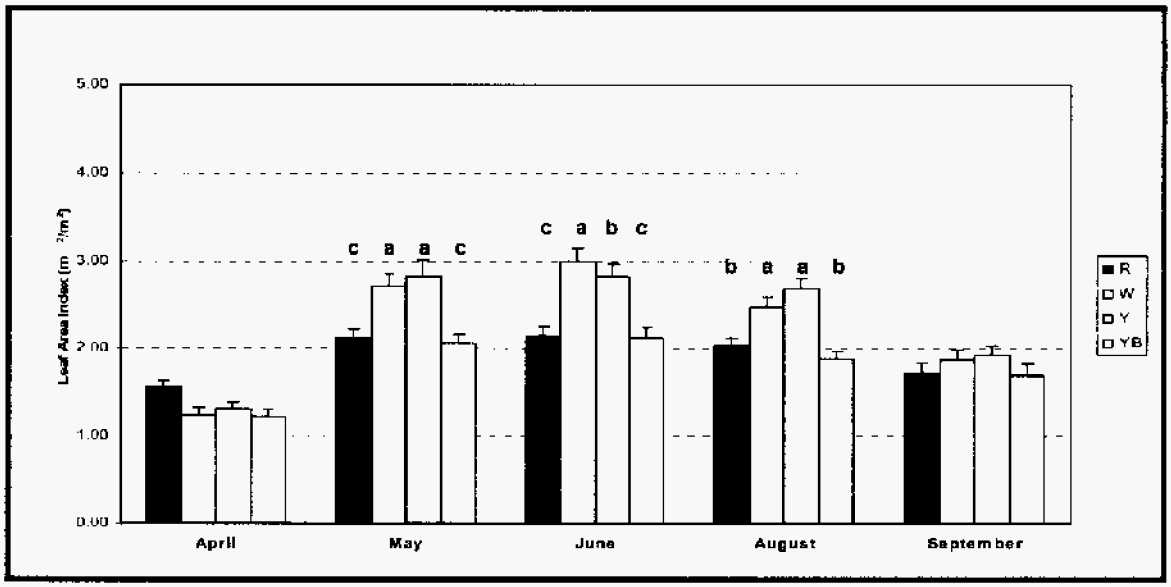

Figure 11. Mean clonal differences in leaf area index in 3-year-old cottonwood grown in a short-rotation intensive culture fertigation experiment near Sumter, SC. Horizontal bars on each treatment mean designate the standard error of the mean. Means within nutrients not connected by the same letter are significantly different at $p \leq 0.05$ based on Waller-Duncan's k-ratio t-test.

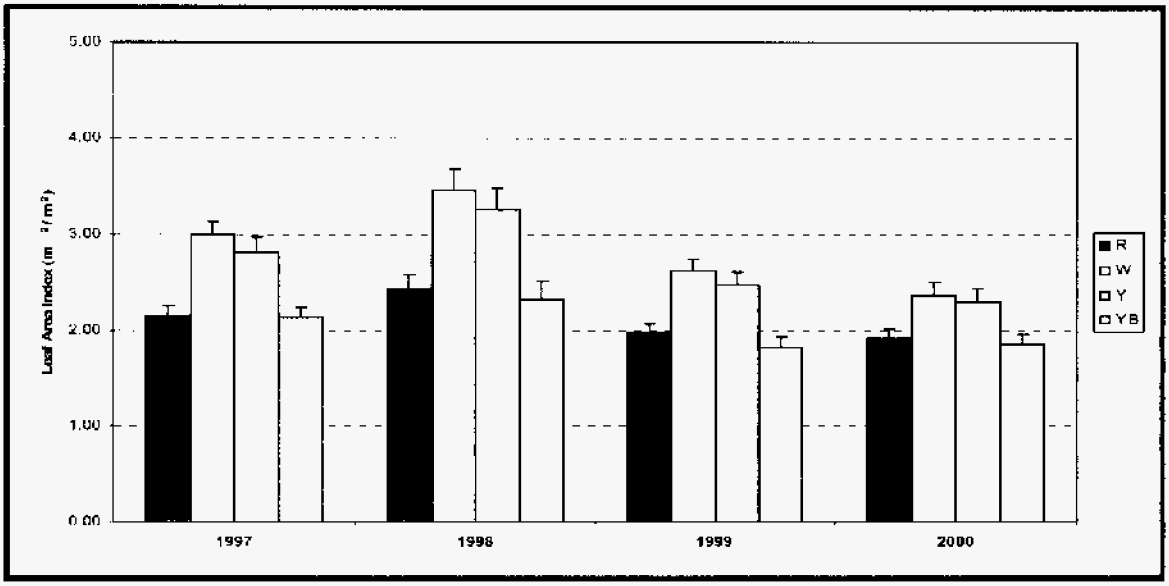

Figure 12. Changes in mean clonal mid-season leaf area index in 3-, 4-, 5- and 6-year-old cottonwood grown in a short-rotation, intensive culture fertigation experiment in Sumter, SC. Horizontal bars on each treatment mean designate the standard error of the mean.

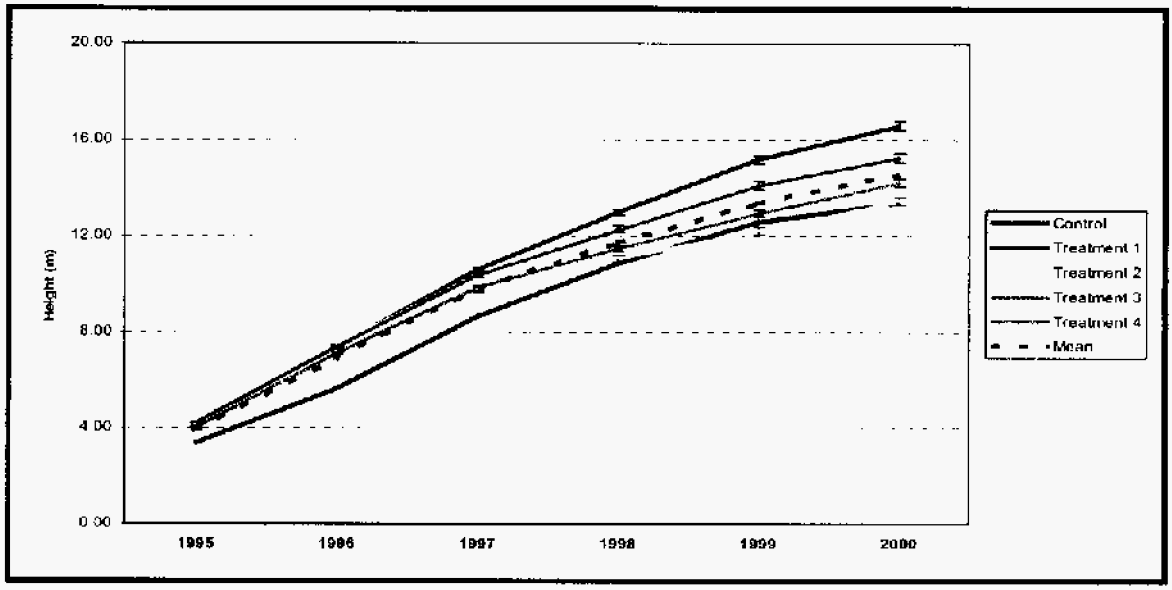

Figure 13. Changes in cumulative height over time in cottonwood grown in a short-rotation, intensive culture fertigation experiment in Sumter, SC. Horizontal brackets at each measurement date represent the standard error of the mean for each treatment. The dashed line represents the overall mean height for all treatments.

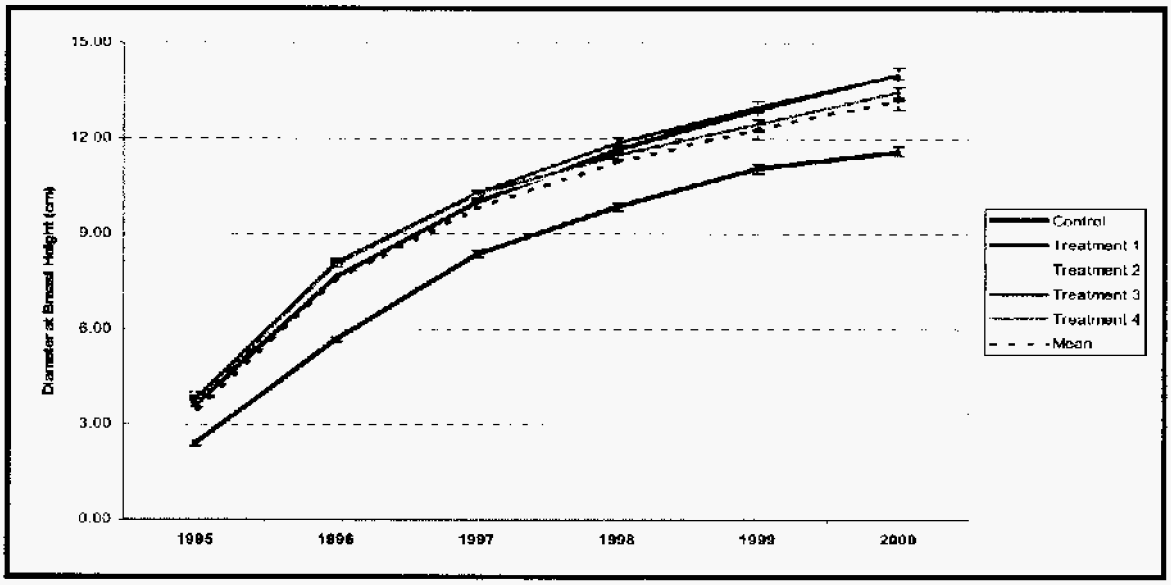

Figure 14. Changes in cumulative diameter over time in cottonwood grown in a short-rotation, intensive culture fertigation experiment in Sumter, SC. Horizontal brackets at each measurement date represent the standard error of the mean for each treatment. The dashed line represents the overall mean diameter for all treatments.

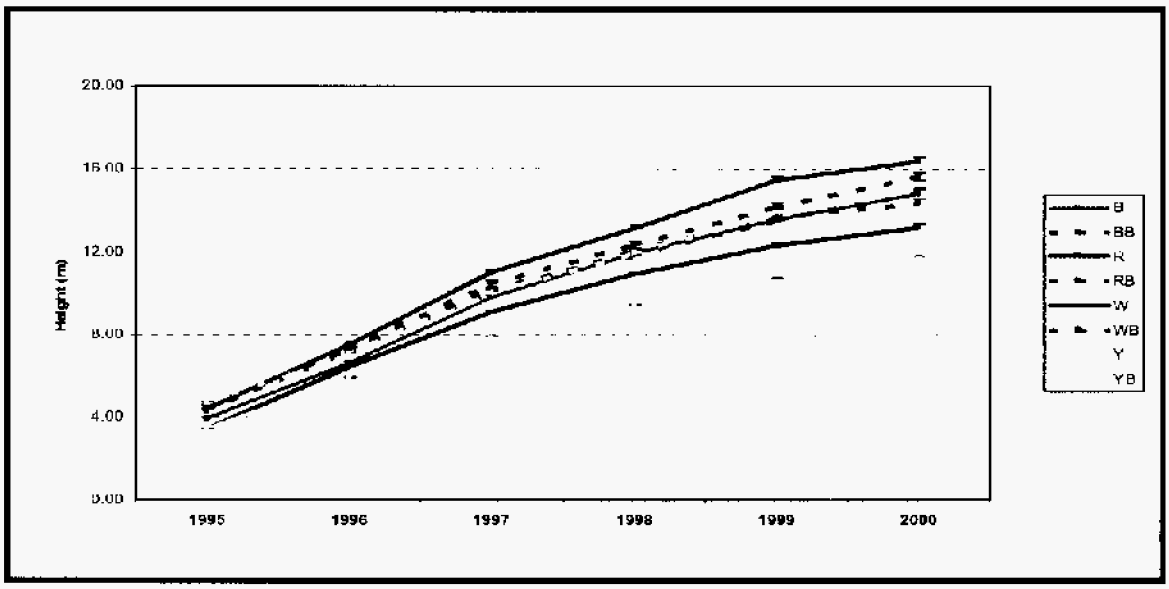

Figure 15. Changes in cumulative height over time in cottonwood grown in a short-rotation, intensive culture fertigation experiment in Sumter, SC. Horizontal brackets at each measurement date represent the standard error of the mean for each clone.

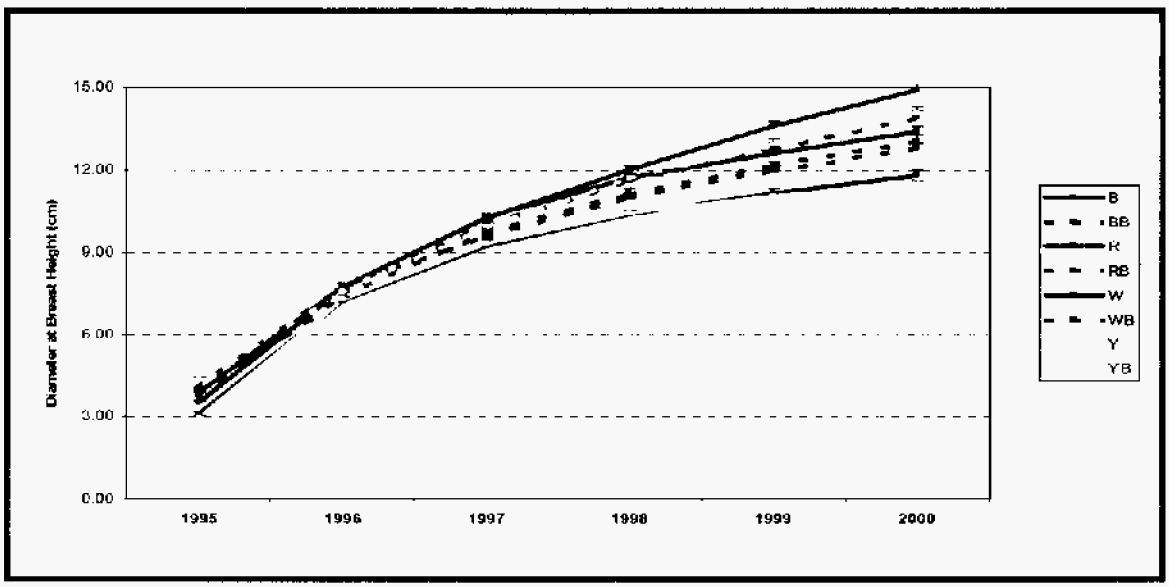

Figure 16. Changes in cumulative diameter over time in cottonwood grown in a short-rotation, intensive culture fertigation experiment in Sumter, SC. Horizontal brackets at each measurement date represent the standard error of the mean for each clone.

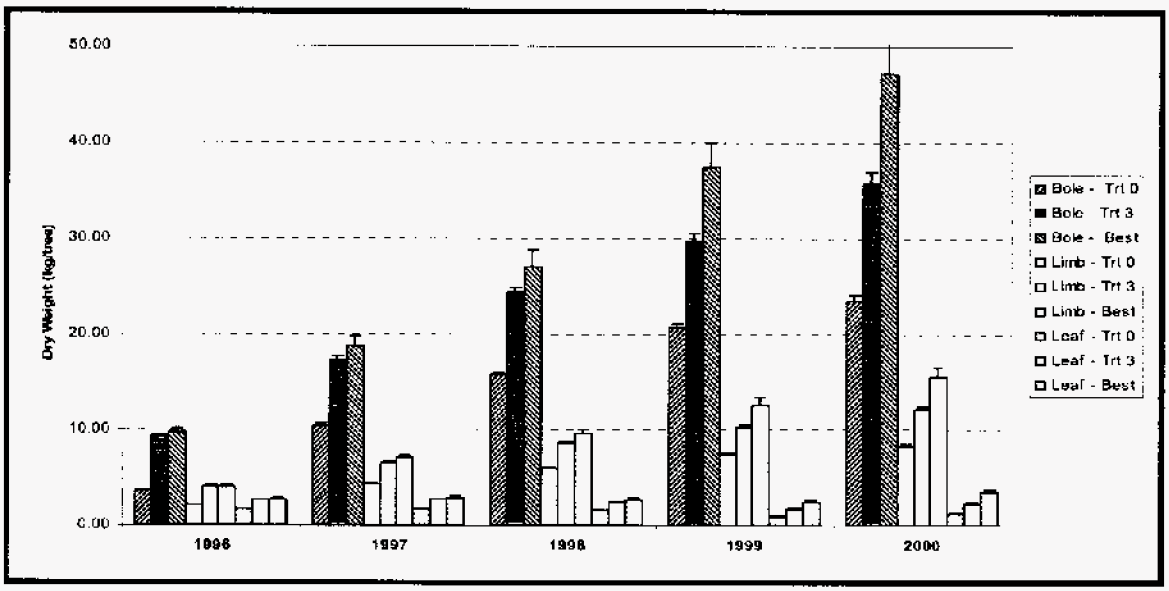

Figure 17. Changes in cumulative above-ground biomass over time in cottonwood grown in a short-rotation, intensive culture fertigation experiment in Sumter, SC. Horizontal brackets at each measurement date represent the standard error of the mean for each treatment.

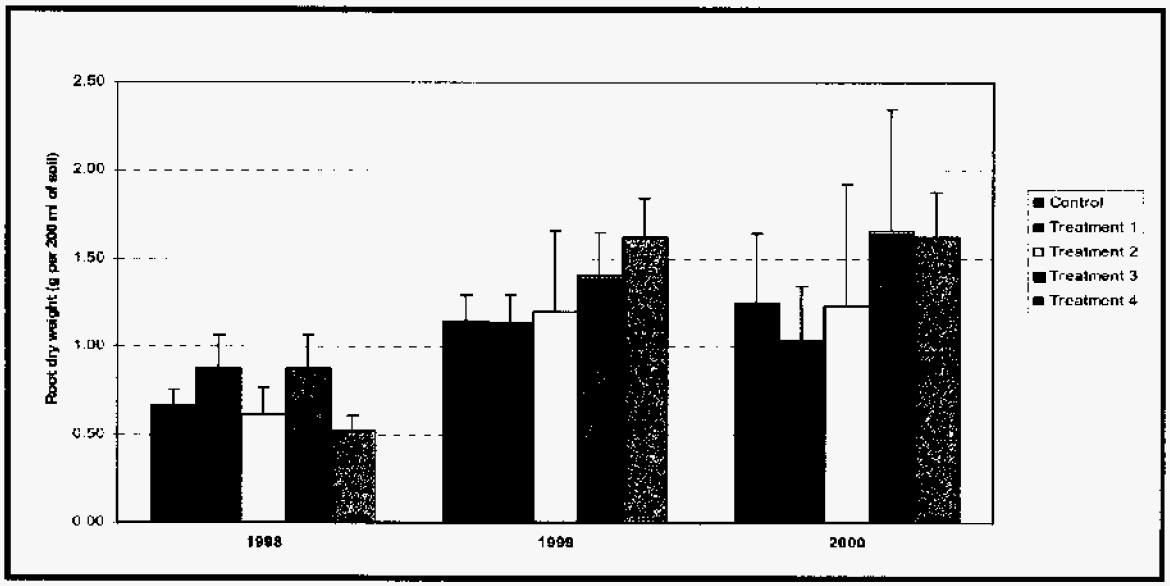

Figure 18. Changes in cumulative root biomass over time in cottonwood grown in a short-rotation, intensive culture fertigation experiment in Sumter, SC. Horizontal brackets at each measurement date represent the standard error of the mean for each treatment.

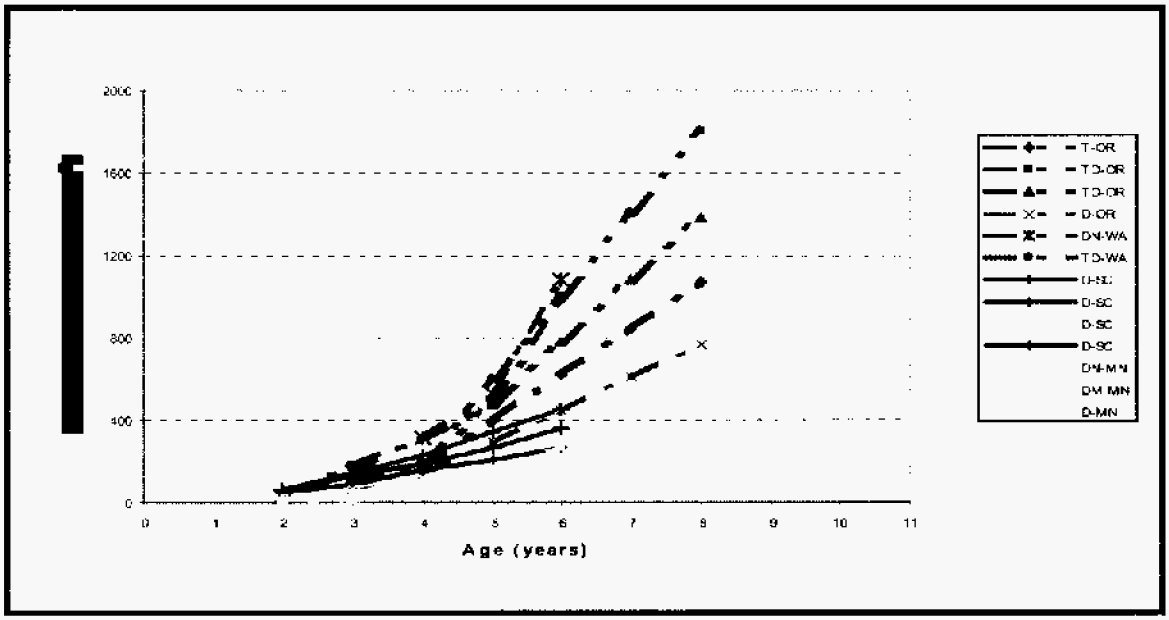

Figure 19. Comparisons among alternate short-rotation experiments involving *Populus* species and hybrids from across the United States. Individual tree volumes are identified by the type of plant material and the state in which the data was collected [T - *Populus trichocarpa*, D - *P. deltoides*, M - *P. maximowiczii*, and N - *P. nigra*].

Table 1. Annual water inputs in cm per species per treatment per year for three hardwood species grown in a short-rotation intensive culture fertigation experiment near Sumter, SC.

	1996	1997	1998	1999	2000
Populus					
Rain	63.25	65.79	67.82	67.82	52.91
High	47.50	44.20	39.88	59.69	53.62
Low	34.80	28.96	25.91	44.20	28.32
Sycamore					
Rain	63.25	65.79	67.82	67.82	52.91
High	44.20	23.88	26.42	54.86	43.43
Low	32.26	19.81	22.35	40.64	28.96
Sweetgum					
Rain	63.25	65.79	67.82	67.82	52.91
High	35.31	38.74	40.13	57.66	45.47
Low	18.29	24.59	23.37	42.16	33.35

Table 2. Annual fertilizer inputs in kg of elemental nitrogen per ha per species per treatment per year for three hardwood species grown in a short-rotation intensive culture fertigation experiment near Sumter, SC.

	1996	1997	1998	1999	2000
Populus					
High	84.63	182.00	136.50	136.50	136.50
Low	42.77	91.00	68.25	68.25	68.25
Control	42.77	65.52	68.25	68.25	68.25
Sycamore					
High	84.63	182.00	136.50	136.50	136.50
Low	42.77	91.00	68.25	68.25	68.25
Control	42.77	65.52	68.25	68.25	68.25
Sweetgum					
High	84.63	136.50	136.50	136.50	136.50
Low	37.31	68.25	68.25	68.25	68.25
Control	42.77	68.25	68.25	68.25	68.25